Quantitative PCR in soil-transmitted helminth programs

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Joint meeting of the BSPP, ISP, BAVP and EVPC

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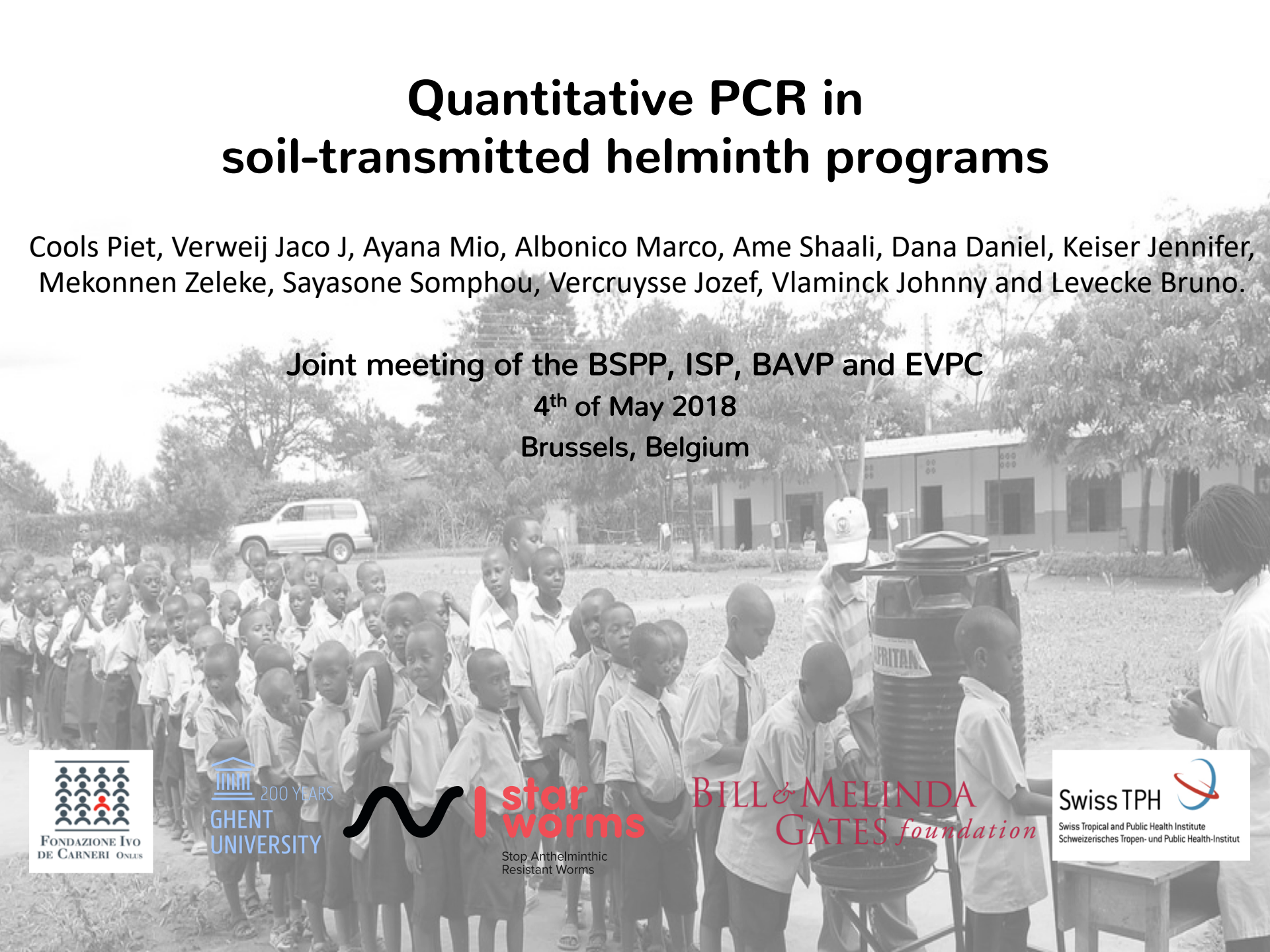
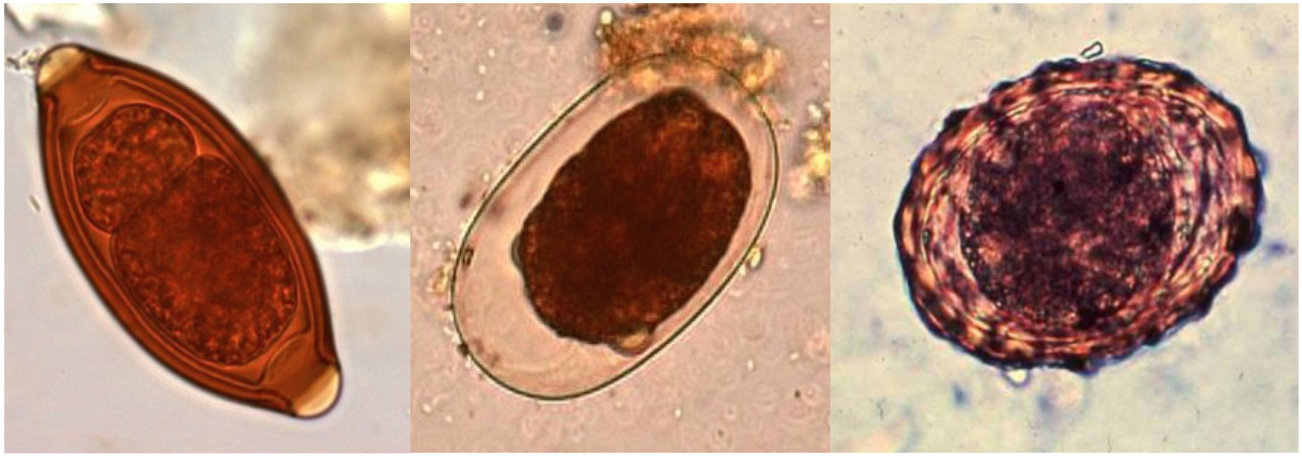
Brussels, Belgium



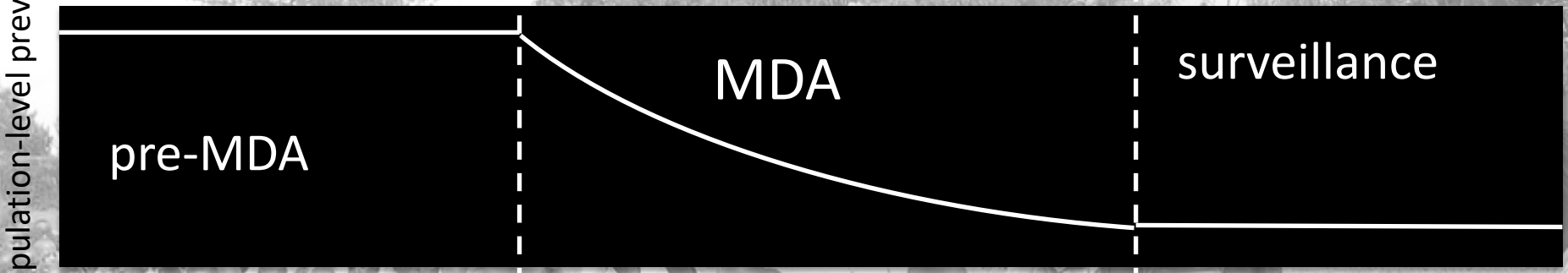
Background

- Soil-transmitted helminths (STHs)
  - *Ascaris lumbricoides*
  - *Trichuris trichiura*
  - hookworm (*Necator americanus* & *Ancylostoma duodenale*)
- STHs > 3 million disability-adjusted life-years worldwide



Background

- Cornerstone control is mass drug administration (MDA)



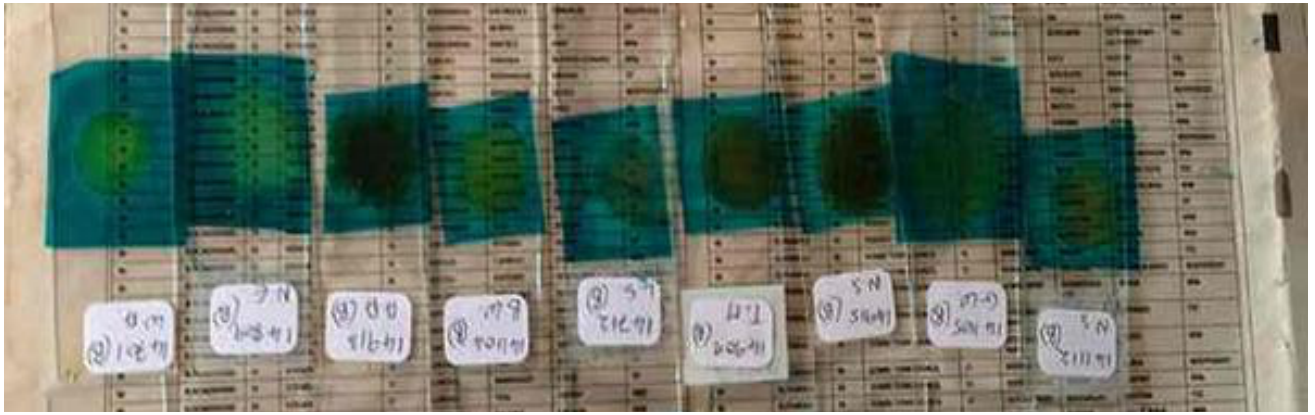
Public health problem?
Which type of MDA?

Drugs working?
Program working?

Stop MDA?

Background

- Diagnostic tools essential in each decision step
- WHO: microscopic detection STHs eggs in stool Kato-Katz smear



Objectives

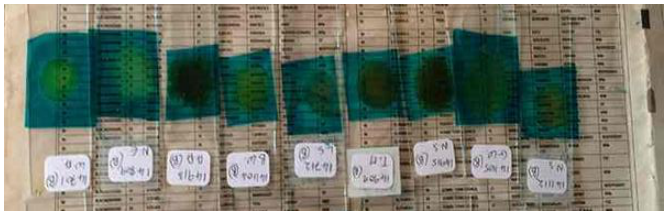
- Investigate applicability of quantitative PCR (qPCR) in STHs programs
  - Sensitivity
  - Drug efficacy
  - Classes of intensity of infection (low, moderate, high)
  - Pooling of stool

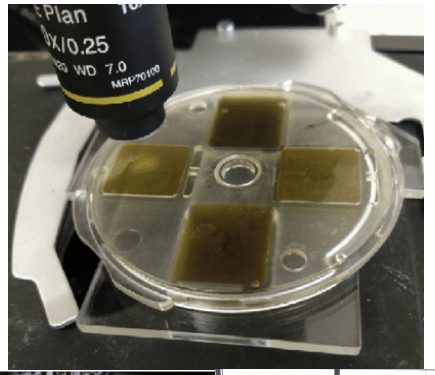
Methodology

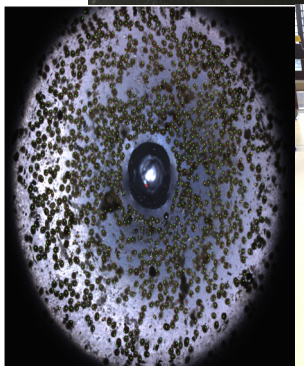
- Drug efficacy trials (Tanzania, Laos, Ethiopia)
- School children baseline and follow-up visit
- Stool ethanol preservation, DNA extraction and qPCR for *A. lumbricoides*, *T. trichiura*, *N. americanus* and *A. duodenale*

Methodology

- Egg counts 4 microscopic methods
  - single Kato-Katz
  - Duplicate Kato-Katz
  - Mini-FLOTAC
  - FECPAK^{G2}







Methodology

- Sensitivity: composite reference method gold standard
- Classes intensity of infection: ROC analysis
- Drug efficacy:
  - Egg reduction rate (ERR) (KK) or genomic equivalents reduction rate (GRR) (qPCR)

$$= 100\% \times \left(1 - \frac{\text{arithmetic mean of group FEC at follow-up}}{\text{arithmetic mean of group FEC at baseline}}\right)$$

  - WHO

	A. lumbricoides	*T. trichiura*	hookworm
Satisfactory	≥ 95	≥ 50	≥ 90
Doubtful	85 < ERR < 95	40 < ERR < 50	80 < ERR < 90
Reduced	≤ 85	≤ 40	≤ 80

- Individual samples vs pools of 10, 20 & 60

Findings: sensitivity

- qPCR is most sensitive method

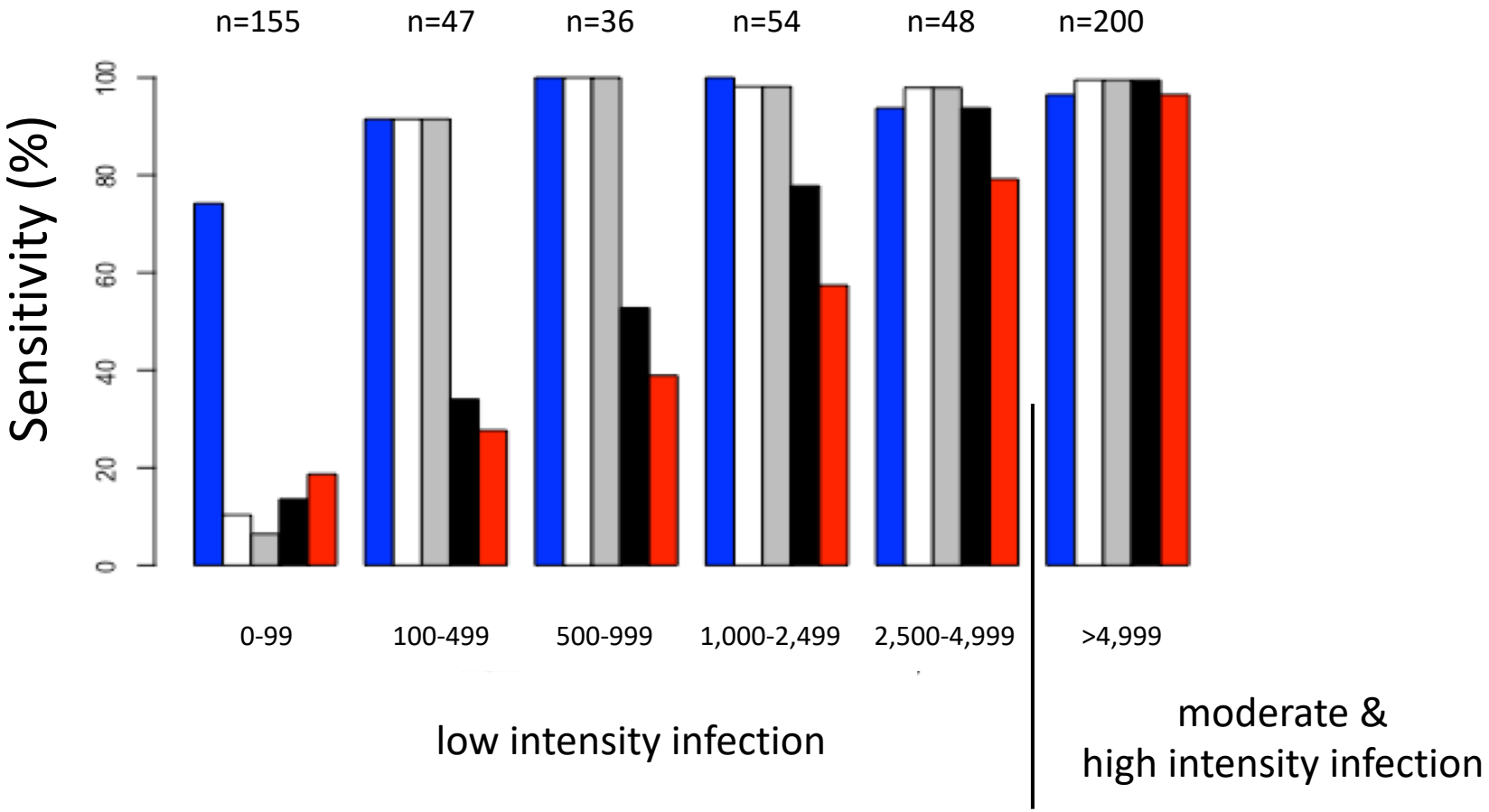
	Ascaris n=540	*Trichuris* n=888	hookworm n=674
qPCR	**90.0** [87.4; 92.4]	**94.7** [93.2; 96.2]	**92.0** [89.1; 93.9]
2x Kato-Katz	**73.0** [69.3; 76.7]**	**91.0** [89.0; 92.9]*	**75.2** [72.0; 78.5]**
1x Kato-Katz	**71.9** [68.0; 75.6]**	**88.2** [86.0; 90.2]**	**72.6** [69.1; 75.8]**
Mini-FLOTAC	**63.3** [59.3; 67.4]**	**91.6** [89.6; 93.4]*	**73.9** [70.6; 77.2]**
FECPAK^{G2}	**58.9** [54.8; 63.1]**	**60.0** [56.5; 63.1]**	**52.4** [48.5; 56.2]**

* $p<0.05$ ** $p<0.001$

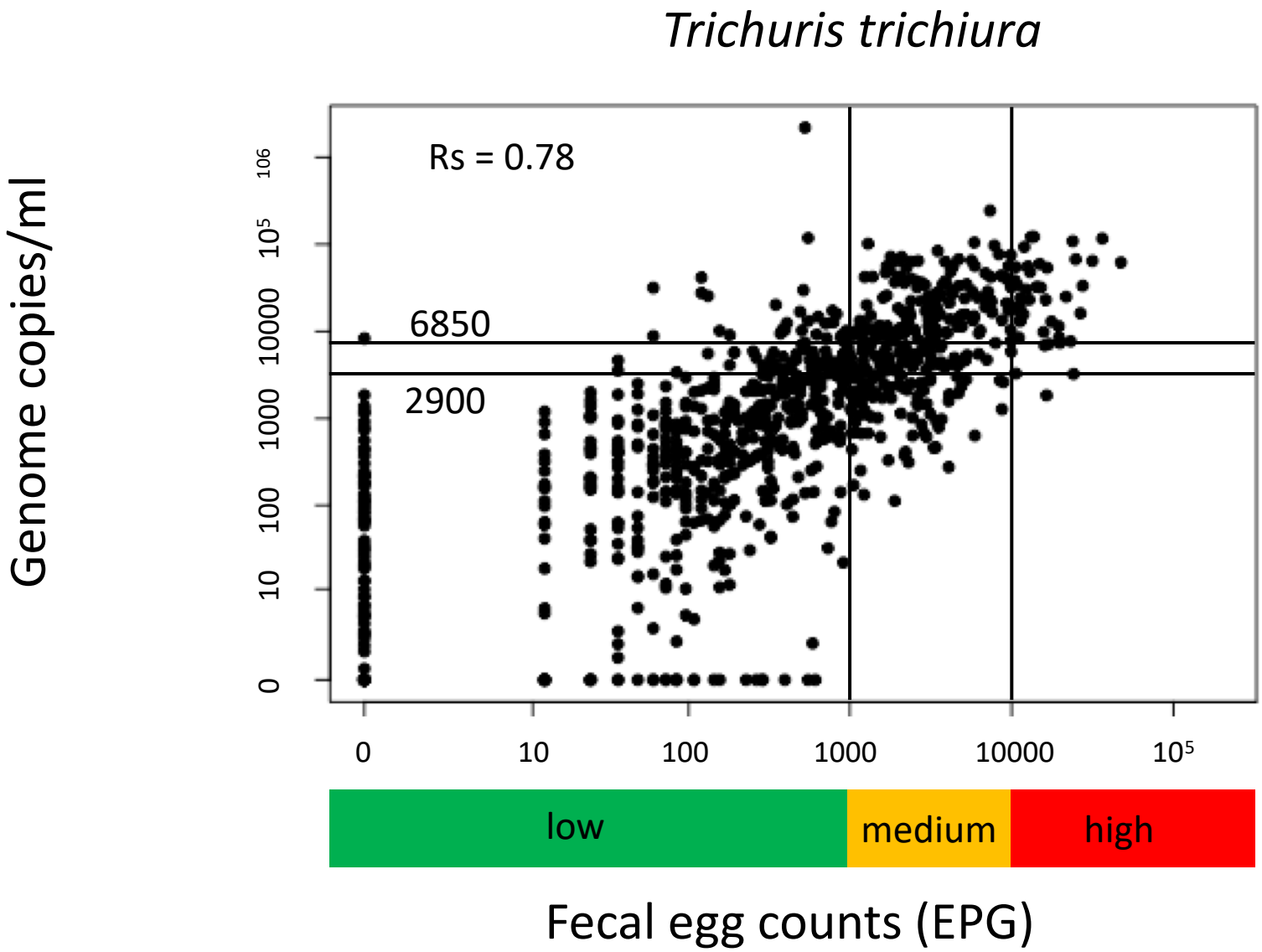
Findings: sensitivity

- qPCR is most sensitive method

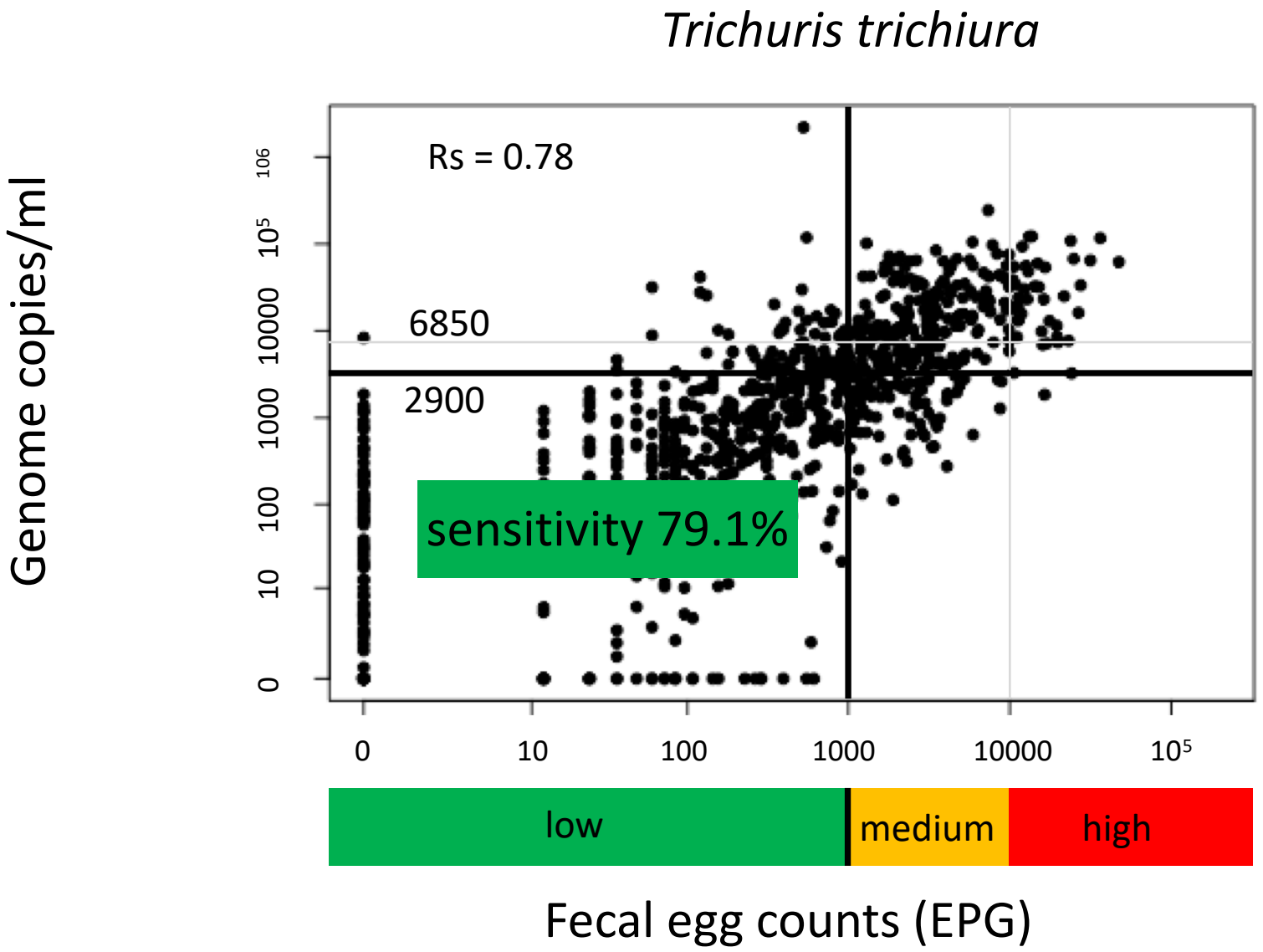
Ascaris lumbricoides



Findings: classes of infection



Findings: classes of infection



Findings: drug efficacy

		Kato-Katz	qPCR
Ascaris	Ethiopia	99.9	99.1
	Laos	99.2	99.1
	Tanzania	96.7	96.8
Trichuris	Ethiopia	48.1	67.5
	Laos	40.5	-4.4
	Tanzania	-4.9	49.0
hookworm	Ethiopia	96.3	94.4
	Laos	96.2	94.6
	Tanzania	83.6	78.1

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normal

doubtful

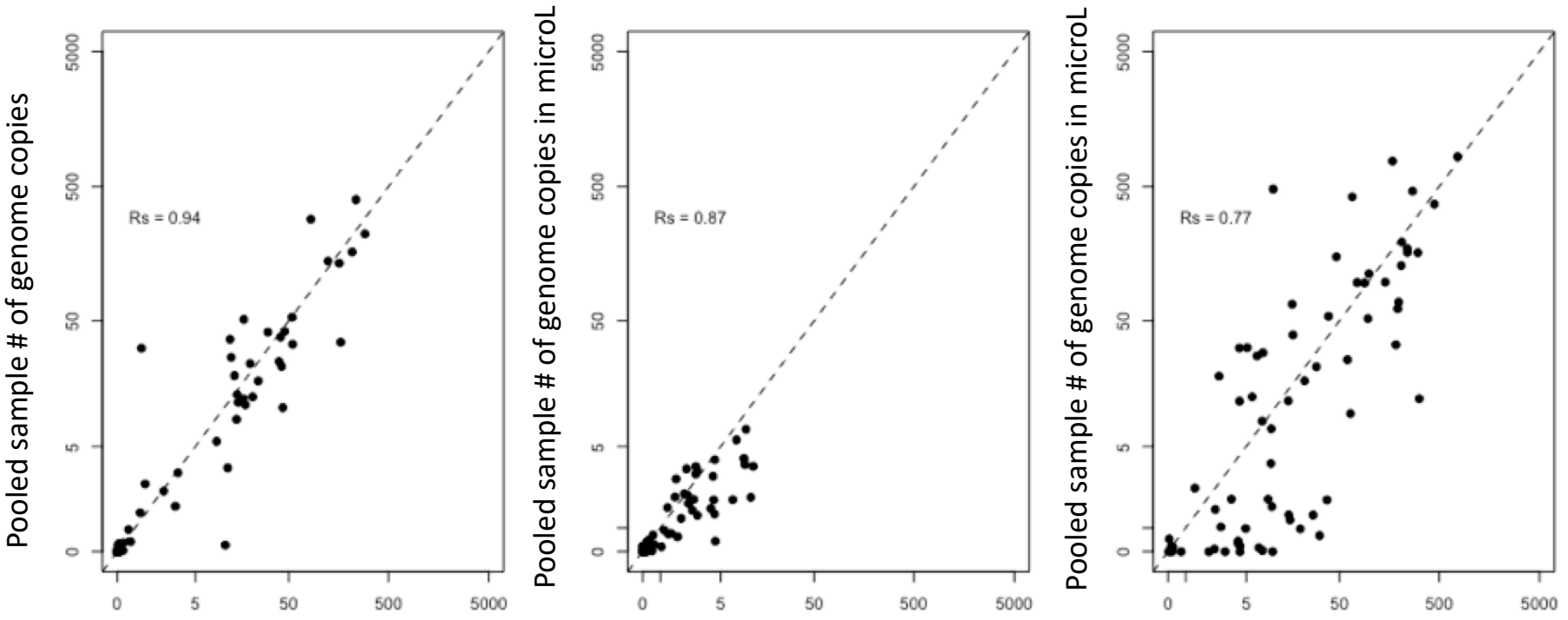
reduced

World Health Organization

ASSESSING THE EFFICACY OF ANTHELMINTHIC DRUGS AGAINST SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHIASES

Findings: pooling

- Pooling holds promise



Conclusions

- Higher sensitivity
- Pooling promising and could reduce costs
- Monitor drug efficacy
- Cost-effectiveness analysis
- Place in STH program: low prevalence/intensity of infection